Qiagen Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit and a Microcentrifuge

1. Inoculate a 5-10 ml LB tube with cells and antibiotic. If desired add 20% w/v glucose to tube to final concentration of 0.2%. Incubate at 37°C for 12-16 hours.
2. (Optional) If necessary, save 800 µl cells along with 200 µl of 50% glycerol in a cryogenic vial after incubation.
3. Spin overnight LB culture in a 15 ml centrifuge tube for 2 min and remove liquid by decanting. Spin again for 1 min and remove liquid by pipetting.
4. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
5. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4-6 times. Do not allow the lysis reaction to proceed for more than 4 min. The suspension should be homogeneously colored blue if LyseBlue has been added to Buffer P1.
6. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. All traces of the blue should have gone and the suspension should be colorless with a white precipitate visible.
7. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge. A compact white pellet will form.
8. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.
9. Centrifuge for 1 min. Discard flow-through.
10. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 1 min.
11. Discard flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.
12. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl of water or Buffer EB to the center of each QIAprep spin column, let stand for 5 min, and centrifuge for 1 min.